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Hydrogen ion concentration

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Hydrogen Ion Concentration and Other Factors Affecting the Distribution of Fucus

by

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Introduction

During the summer of 1918 the writer worked on the effect of light and desiccation on the distribution of *Fucus evanescens* Agardh. In places, however, little or no Fucus was found where there was sufficient light and where desiccation to any considerable extent did not occur. Fucus frequently did not occur in bays which had light as well as suitable places for attachment. The writer has never found growing Fucus in tide-pools, although some were completely surrounded by it. Fucus does not grow where Ulva is found in any considerable quantity. Both Fucus and Ulva utilize the CO_2 in the sea-water during the day in the synthesis of their carbohydrates, thus causing the water to become more alkaline. During the night CO_2 is given off, thus causing the water to become more acid. Animals also add to the acidity of the water. Tests showed the hydrogen ion concentration to be much higher where Ulva grew than where Fucus grew.

Historical

Little work has been done on the factors causing the distribution of Fucus. Davis (1911) says the depth to which certain algae may descend depends upon the penetration of light. The factor that determines the lowest limits of algal life is not the depth of the water but the absence of light. The green algae require the most light, the red the least and the browns are intermediate. The same writer also considers that the influence of temperature must be of fundamental importance in the distribution of algae where the seasonal extremes are as great as those of the summer and winter at Woods Hole. There Fucus is found in its best vegetative condition during winter and spring, fruiting most abundantly during the latter season. It is represented during the summer by dwarfish growths, frequently lighter in color than the winter condition in which the growth and fruiting is more uniform. Most of the winter growth matures during the spring, hence the display of Fucus during the summer is comparatively poor.

Gail (1918) found that light and desiccation are controlling factors in the distribution of Fucus.

Little work has been done dealing with the effect of hydrogen ion concentration on plants, and no literature was found dealing with its effect on Fucus. Gillespie (1918) found the growth of the potato scab *(Actinomycoses chromogenus)* in media at the exponent 5.2 was slower and generally less vigorous than at less acid exponents. Sometimes the strain succeeded in growing well in a medium which had initially an exponent of 5.2 or even 4.8, but the growth was accompanied by a marked decrease of acidity, and the manner of the growth gave reason to doubt whether even in these cases more than a poor growth can occur in such exponents.

Cohen and Clark (1919) find in several species of Bacillus that there is a broad zone of pH within which the rates of growth are quite uniform for these short periods during which the increase of viable cells approaches the logarithmic rate. On borders of these zones of pH slight change in the pH produces marked effect upon reproduction.

Itano and Neill (1919) report that *Bacillus subtilis* germinates at 25° and 37° C if the hydrogen ion concentration of the broth is kept between pH 5 and pH 10 but not higher or lower pH values.

Shelford (1918) found that death among young herring occurred after an exposure of 8 hours when the pH was brought down to 6.825 or just on the acid side of true neutrality. Lillie, Loeb, Medes and Moore have gotten similar results on sperms and newly fertilized eggs.

Methods and materials

Experiments were started in June, 1919, at the Puget Sound Biological Station to determine if the hydrogen ion concentration had any bearing on the distribution of Fucus.

The salts used were potassium acid phosphate (KH_2PO_4), boric acid (H_3BO_3), potassium chloride (KCl) and sodium hydroxide (NaOH). The first three salts were recrystalized from 3 to 5 times. In all essentials the methods used were those of Clark and Lubs (1917).

The sodium hydroxide was prepared from metallic sodium. A piece larger than would be required was placed in a paraffined bottle which contained sufficient conductivity water to cover the sodium. The mouth was closed with a paraffined stopper. The solution was poured off and discarded when the outer layer had dissolved and approximately the desired amount of the unoxidized sodium remained. At once, and as rapidly as possible, unused conductivity water was poured on the sodium in the paraffined bottle and the mouth was closed with a paraffined stopper. After 24 hours, when the sodium had thoroly interacted with the water, the desired normality of the solution was obtained by adding a sufficient amount

of conductivity water and titrating with benzoic acid 99.90% pure fused, Bureau of Standards. All other solutions, including the buffers, were made according to the methods of Clark and Lubs (1917). These solutions were also kept in paraffined bottles or resistance glass.

The conductivity water was prepared by the distillation of barium hydroxide (Ba $(OH)_2$) sat. sol. 100cc, barium chloride ($BaCl_2$) sat. sol. 75 cc, alkaline potassium permanganate sol.* 50 cc, and enough distilled water to make 2 liters. The first and last 600 cc of distillate were discarded. The conductivity water and buffers were tested with the calomel cell.

The indicators used were phenolsulphonphthalein and o-cresolsulphonphthalein. A hydrogen set put up by Hynson, Westcott and Dunning of Baltimore was also used, and proved very helpful as a check.

pH Determinations

Tests were made on alternate days for the hydrogen ion concentration during the last half of June, thru the entire month of July, and thru the greater part of August. They were made in the following locations:

I. Places where Fucus was abundant.

1. Sunny exposure on Brown Island.
2. Southeast half of Turn Rock.
3. Southeast exposure on San Juan Island opposite Madrona Point.
4. On beaches having good rock for attachment of Fucus, much light and little or no Ulva.

II. Places where little or no Fucus grew.

5. On beaches having good attachment, good light and an abundant growth of Ulva.
6. In tide-pools containing no Fucus but with an abundant growth of it about them.
7. Out in the Sound away from visible vegetation.
8. On beaches having poor attachment for Fucus, much light and little or no Ulva.
9. Locations having little or no direct sun light.

* NaOH 200g., KMnO, 8g., distilled in H_2O made up to 1000 cc.

TABLE 1. *Average monthly pH values of seawater June 15 to August 15, 1919.*

	June		July		August		Av. for Per.	
Time of Testing	a.m. 5-6	p.m. 1-4	a.m. 5-6	p.m. 1-4	a.m. 5-6	p.m. 1-4	a.m. 5-6	p.m. 1-4
Location	*Much Fucus*							
1 Southeast exposure	7.8	8.37	7.65	8.15	7.73	8.35	7.72	8.31
2 " "	7.81	8.3	7.8	8.26	7.8	8.15	7.80	8.23
3 " "	7.8	8.175	7.77	8.23	7.75	8.25	7.77	8.21
4 Beach, good attachment	7.6	8.0	7.72	8.27	7.53	8.2	7.62	8.16
	Little or no Fucus							
5 Beach, Ulva present	7.71	8.60	7.56	8.73	7.80	8.80	7.69	8.71
6 Tide pools	7.41	8.45	7.23	8.62	7.66	8.70	7.43	8.59
7 Out in Sound	7.90	8.00	8.00	8.16	8.14	8.25	8.01	8.15
8 Gravel beach; no Ulva	7.90	8.00	8.10	8.20	8.00	8.15	8.00	8.12
9 Little direct sunlight	7.91	8.13	7.84	8.15	8.03	7.97	7.92	8.07

Table 1 shows the different pH values of the seawater and the locations are referred to by numbers which correspond to those above. The pH value of the locations having much Fucus does not vary much *either* side of 8.0 in the afternoons. The average difference between the forenoon and the afternoon is 0.50. In case of tidepools and of beaches having much Ulva, but neither having Fucus, the pH values of the water in the afternoons is considerably above that of 8.00. The average difference between the forenoon and afternoon is 1.09, which is much greater than the average difference where much Fucus is found. This would seem to indicate that the pH values of the water were not favorable since all other conditions were good.

The last three locations, namely (7) out in the Sound away from visible vegetation, (8) on beaches having no Ulva and (9) on exposures receiving little or no direct sunlight, have pH values well within the limits for a good growth of Fucus. They have an average difference between the morning and afternoon of only 0.035. There must be other factors which prohibit the growth of Fucus in these locations, and these factors will be considered in a later part of this paper.

Effects of Ulva on Fucus

To study the effect that Ulva might produce on Fucus, 2-4-lobed plants, and more mature ones but not fruiting, with their natural attachments, were selected. These were placed in the Sound, where much Ulva was growing, others in Fucus beds. All plants were apparently of equal vigor. A stout cord was tied to each rock containing the Fucus plants which were placed among the Ulva. To the other end of each cord a piece of wood was tied. The cord was of sufficient length to permit the wood to float at high tide. Thus the Fucus could readily be found. The rocks containing the Fucus which was placed in the Fucus beds were suffi-

ciently heavy to hold them in place, and they were less difficult to find than among the Ulva. There were six rocks bearing the 2-4-lobed Fucus plants and the same number bearing the more mature plants among the Ulva. The same number of each kind were placed in the Fucus beds. All were placed about 1.5 meters vertically above the —1 tide line.

After three weeks both the young and mature Fucus plants placed among the Ulva had become much darker in color, and by the end of 4.5 weeks they had a decidedly reddish tinge. This is an abnormal color. At the end of 11 weeks the reddish tinge was still present except for a space of 3.5 mm around the tip ends and margin of the thallus where they were very dark thruout. A microscopic examination of sections thru the thallus showed a very evident gradient of susceptibility. The cells at the margin were dark brown in color and the walls were in a state of collapse. A little farther in they were still intact but the protoplasm was very dark. In cells still farther in, the protoplasm was more normal in color and normal chloroplasts could be detected. The plants had the same number of lobes that they had in the beginning of the experiment. Measurements showed that on the average they had not increased in size.

Both the young and the mature plants placed in the Fucus beds had normal color at the end of 11 weeks. The young plants placed in the Fucus bed had grown to twice the size of the young plants placed among the Ulva. A microscopic examination showed the cells to be normal.

The average pH value of the water about the Ulva at about 6:00 A. M. was 7.85, and at about 2:00 P. M. was 8.72. The average pH value of the water about Fucus at about 6:00 A. M. was 7.88, and at about 2:00 P. M. was 8.21. The average temperature of the water among the Ulva was 13° C at about 2:00 P. M. The average lower extreme was 11° C. The light was of the same intensity since they were the same distance above a —1 tide and were on the same exposure. All conditions seemed to be the same except that of the pH value of the water. The difference in growth and color in this experiment points toward the difference in the pH values of the water in the two locations, as a means by which the presence of Ulva may limit the distribution of Fucus.

Effect of pH and temperature on young plants

Experiments were started to study the effect on Fucus plants of pH values on the acid side of true neutrality and on up thru the higher alkaline values.

Open 4-liter glass jars were used, and three liters of water were used in each jar. The water was taken from the Sound, away from visible vegetation. It had an average pH value of 8.05. Commencing with pH value of 6.6 each jar was labeled respectively 6.8, 7.0, 7.2, etc., thru 8.8.

The correct pH value from 6.6 to 8.0 was secured by the addition of HCl. From 8.2 thru 8.8 the correct pH value was secured by the addition of sufficient amounts of water from about Ulva. When the tide was high it was necessary to add NaOH in place of water taken from about Ulva. In the addition of both the HCl and NaOH a medicine dropper was used. The indicator used for testing the pH values was phenolsulphonphthalein. The hydrogen ion set was used wholly for the comparison of colors. In those cases in which the pH values ranged from 6.6 to 8.0 it was necessary to make the pH corrections of the water nearly every hour of the day. The number of changes necessary depended largely upon the intensity of the sunlight. The correction of the pH values above 8.0 was necessary two or three times per day depending also upon the intensity of the sunlight. These changes kept the pH values of the water reasonably accurate. The seawater was changed completely in each jar once each day.

The temperature of the water in two of the sets of the experiment varied from 11° C to 24° C. These were the temperatures secured on the float where the jars remained during the time of the experiment. The temperature of the water in the third set varied from 10.5° to 13° C. This temperature was obtained by suspending in the Sound trays with bottoms of wire netting. The trays were fastened by cords between two logs on a float. The jars were placed in the trays. The trays were regulated in such a manner that the surface of the water in the jars had the same level as the surface of the water of the Sound. The lower temperature, 10.5° C, was the average temperature of the water in the jars at night. The higher temperature, 13° C, was the usual temperature at about 1:00 P. M., which was about 1° C higher than the temperature of the Sound at that time. Two-lobed young Fucus plants with natural attachments were used. Three such sets were continued over a period of nearly 11 weeks. All conditions were the same except that of temperature.

The results of the two sets having the temperatures range from 11° C to 24° C will be considered first. The Fucus plants in the seawater having a pH value of 6.6 and 6.8 lived about 9 days. The thalli of the plants commenced to curl on the second day. They showed signs of whiteness about the margins on the third day. On the fifth day the larger portion of the entire thallus looked whitish and soft. A microscopic examination of the cells on the seventh day showed those near the tips and margins to be completely broken down. The cells a little farther in were very much plasmolyzed. Still farther in, a few of the cells were apparently normal. No cells were normal on the ninth day. Here also the gradient of susceptibility is well shown. These plants were kept two weeks longer in the seawater having their respective pH values. At the end of this time

practically all of the cells were broken down and the plants were becoming frayed. The effect on the Fucus plants in seawater having pH values of 7.0 and 7.2 was similar, but three or four weeks were required to bring about the same results.

Those plants in seawater having pH values of 7.4, 7.6 and 7.8 showed little or no effect except that the growth was somewhat inhibited. There appeared to be a physiological adjustment to some extent, at least temporarily. Since Fucus is rarely found in seawater having a pH value as low as 7.4, and this for only a short period in the early morning, it would seem that it might be a matter only of time until the plants in seawater having this pH value and possibly the plants in seawater having a pH value of 7.6, would show results similar to those in the lower pH values.

The plants in seawater having a pH value of 8.0 to 8.2 had increased on the average 1 cm in length, and about 80 per cent of them were 4-lobed. In one jar containing seawater having the same pH value, the Fucus plants had not grown as much. These had increased in length only about 7 mm but the same percent of them were 4-lobed. There was no cause apparent for this difference since the conditions were the same and they had received the same treatment. There may have been some difference in the vigor of the plants but they appeared the same at the beginning of the experiment. At the end of 11 weeks the experimental plants were compared with those on the shore from which they were taken. The growth was practically the same. Those in the jars having seawater with a pH value of 8.0 to 8.2 were slightly lighter in color. The cause was undoubtedly due in a large degree to the difference in temperature, as will be explained later.

The Fucus plants in seawater having a pH value of 8.4 became abnormally darker after about 4.5 weeks and remained darker thruout the entire time. They showed an average increase in length of 3 mm at the end of 11 weeks. Two of the 30 plants in the jars containing seawater having a pH value of 8.4 had become 4-lobed.

The Fucus plants in seawater having pH values of 8.6 and 8.8 became very dark at the end of the first week. They also appeared more leathery and soon took on a reddish tinge. A microscopic examination after 8 weeks showed all the cells to be dead, except those in the interior part of the thallus. The protoplasm was brownish in color and many of the cell walls near the outer margin were collapsing. No growth had taken place at the end of 11 weeks and very few cells appeared normal.

The results from the set having the temperature range from 10.5° to 13°C will now be considered. In jars containing seawater with pH values of 6.6, 6.8 and 7.0 the results were practically the same as in the two previous sets except in degree and in that a longer period of time was required. No effects were noticeable until on the 8th day on the Fucus

in seawater having a pH value of 6.6, and none until at the end of three weeks on those in 6.8 and 7.0.

No effects could be seen in 7.2, 7.4 and 7.6 except that growth appeared somewhat inhibited. In 7.8 there was an average increase in length of 5.5 mm, and some showed the beginning of four lobes.

The plants in seawater having pH values of 8.0 to 8.2 had on the average the same growth as the plants in the same pH values but with the temperatures ranging from 11° to 24°C. The color, however, was more nearly the same as the color of the Fucus plants of the same age growing on the shore. This wide range of pH values and higher temperature must account to considerable extent for the lighter color of the Fucus plants in the previous set having the same pH values.

The Fucus plants in the seawater having pH values of 8.4, 8.6 and 8.8 were affected in practically the same manner as in the previous set with the higher and wider range of temperature. The period of time necessary to show the same results was from 4 to 6 days longer in this narrow range and lower temperature.

Percent of germination of oospores

Experiments were also undertaken with a view to determining effects both of temperature and of pH values of the seawater on the acid side of true neutrality and on up through the higher alkaline values, upon the germination of oospores and upon the subsequent growth of the sporelings produced. The oospores were obtained in the manner previously described (Gail, 1918). They were germinated on microscopic slides placed in glass jars containing seawater having the different pH values. Four different ranges of temperature, 10.5°-13°C, 11°-17°C, 11°-24°C and 11°-30° were used. The temperature of 10.5°-13°C was secured by suspending trays with bottoms of wire netting in the Sound as was previously described. The temperature of 11°-17°C was secured by placing the jars containing the different pH values of seawater in porcelain pans containing seawater, and setting these on a float on the Sound. The pans were about 60 cm high. The water in the pans was changed as often as was necessary to keep the temperature at 17°C or below. The temperature of the seawater having the different pH values usually went down to 11°C at night on the float. The temperature of 11°-24°C was the temperature produced by the atmosphere on the float some distance from the bank. The last temperature, 11°-30°C, was that produced by the atmosphere on the float but near the shore where it was protected from the wind. Each set was run in duplicate.

The per cent of germination will be considered first. An examination of table 2 will show the following:

TABLE 2. *Percentage of germination in different pH values and temperatures of water.*

pH	10.5°-13°C	11°-17°C	11°-24°C	11°-30°C
8.8	30	12	13	0
8.6	75	60	40	15
8.4	90	90	67	20
8.0-8.2	95	92	85	20
7.8	80	71	75	13
7.6	75	82	49	10
7.4	60	60	41	3
7.2	40	28	15	0
7.0	40	31	3	0
6.8	36	10	5	0
6.6	32	5	0	0

1. The higher percent of germination occurs in seawater having pH values above 7.4 and below 8.6 in all temperatures considered. The maximum germination occurs in seawater having pH values between 8.0 and 8.2.

2. The per cent of germination usually diminishes above a temperature of 17°C.

3. A microscopic examination of the oospores in seawater having pH values above 8.2 with a temperature above 24°C showed that the cell wall and plasma membrane were ruptured and that the protoplasm was exuding.

PERMEABILITY OF OOSPORES

Experiments were now made to determine whether permeability is related to inhibition of germination of oospores and to the death of oospores in different pH values of seawater. Oospores were secured in the usual manner. These were stained one to three hours in a weak neutral red solution. Watch glasses were washed in seawater and rinsed thoroly with seawater having the pH value that was to be used in that particular watch glass. The watch glass was then labeled with the correct pH value. Watch glasses for each pH value used were treated in the same manner. A large number of stained oospores were now placed in each watch glass by means of a medicine dropper. The medicine dropper was also used to place seawater having correct pH values on the stained oospores. The water on the oospores was changed four times. This was done to make certain that the correct pH value was in each glass, as the oospores were stained in seawater having a pH value of 8.2.

During the first attempts the oospores were examined every 15 minutes under a compound microscope to observe any change. As no

changes could be detected, the time for the examination was extended to about one hour. Decided changes in color usually occurred after about 12 hours. Each oospore is covered with a gelatinous substance which probably accounts for the long period of time required before any definite changes resulted. In no case did a change in color take place in all oospores.

Eighty per cent of the oospores in seawater having pH values of 6.6 and 6.8 changed from red to darker red or purple. No change of color occurred in oospores which were in seawater having pH values between 6.8 and 8.2. This indicates that substances potentially acid or alkaline do not permeate the plasma membrane in sufficient quantities to change the color produced by staining the oospores with neutral red. Oospores in seawater having pH values below 7.6 were not killed but were inhibited when the temperature was below 24°C. This was shown by the fact that when the pH values were allowed to rise, germination and some growth took place. When the temperature of the water was above 24°C the oospores were killed in 3 to 24 hours, depending upon the height of the temperature. Better germination occurs in seawater having pH values between 7.4 and 8.4. The maximum germination is at about 8.0 or 8.2 and gradually decreases on either side. This is in accord with the observations that the plasma membrane was not permeated by OH or H ions in any considerable quantity in these pH values as is manifest by no change in color. In seawater having pH values of 8.4, 8.6 and 8.8 about 70 per cent of the oospores changed from red to yellow. Usually there was a considerable space between the cell wall and the protoplast. The OH ion had probably produced a sufficient disturbance of the colloidal equilibrium of the plasma membrane to bring about plasmolysis. Harvey (1911) reports injury to be possible when the concentration of the base was 0.025N.

Plate 51 shows a graphic representation of the germination during the first 7 days at different temperatures and the various pH values of the seawater.

Growth of sporelings

Oospores were germinated in the usual manner and the sporelings were grown for four weeks. The same conditions and manner of treatment were continued that were used in the study of the per cent of germination of oospores. The percentage of living sporelings is based on the number of oospores that had germinated by the seventh day. Table 3 shows the percentage of living sporelings at the end of four weeks together with the pH values of the water and the variations in the

temperature. With a few exceptions there is a gradual decrease in the number of living sporelings as the temperature becomes higher than 17°C. The percentage of living sporelings is very small when the temperature reaches 30°C. The maximum number is nearly always found in water having pH values of 8.0 to 8.2. For a graphic representation see plate 51.

TABLE 3. *Percent of living sporelings at the end of 4 weeks, in different temperatures and pH values*

pH	10.5°C-13°C	11°C-17°C	11°C-24°C	11°C-30°C
8.8	0	0	0	0
8.6	29	30	22	0
8.4	56	83	70	45
8.0-8.2	96	98	81	5
7.8	86	90	82	3
7.6	85	91	81	4
7.4	52	40	26	0
7.2	45	28	7	0
7.0	45	29	0	0
6.8	20	8	2	0
6.6	19	3	0	0

The size of the sporelings growing in seawater having the various pH values and a temperature ranging from 11°-17°C were recorded at frequent intervals. Both the length and the width of the sporelings were measured. The increase in width is very small during this period of growth and will be considered later in this article. Each measurement recorded represents the average length and width of 12 typical sporelings. Very little growth occurred in seawater having a pH value of 6.6. The sporelings were still alive at the end of four weeks, as they increased in length from 0.104 mm to 0.16 mm when the pH value of the sea water was raised from 6.6 to 7.4.

The growth of sporelings in seawater having pH values of 7.0 and 7.2 was almost completely inhibited after the eighth day.

The growth in seawater having pH values of 7.4, 7.6 and 7.8 was very much the same. Growth was inhibited to some extent after about two weeks.

No inhibition of growth is apparent in seawater having pH values of 8.0-8.2. Numerous sets of experiments showed the same results in all temperatures tried below 24°C. The sporelings increased in length from 0.072 mm to 0.192 mm in 72 hours. This was an increase of .12 mm. By the eighth day the length was 0.404 mm. Growth continued thruout the period.

The sporelings in seawater having a pH value of 8.4 increased from 0.072 mm to 0.224 mm in the first 72 hours. This was an increase of 0.152 mm, which was a greater increase than occurred in seawater having a pH value of 8.0-8.2. By the eighth day the length was 0.30 mm, and by the thirteenth day it was 0.332 mm, an increase of only 0.032 mm in five days, while the sporelings in the seawater having pH values of 8.0-8.2 were 0.40 mm long in the same time. Growth seems to be inhibited after about 72 hours in seawater having a higher pH value than 8.2.

The sporelings growing in seawater having a pH value of 8.6 were living at the end of four weeks. At this time they were 0.24 mm in length, an increase of 0.178. The growth was quite rapid for the first eight days, when it was practically inhibited, and many appeared dead by the eighteenth day. A considerable number of the sporelings became loosened from the slide during the remainder of the four weeks.

The sporelings growing in seawater having a pH value of 8.8 grew very slowly and life was considered extinct after the seventh day. No growth took place after this time and the sporelings were dark gray instead of brown in color. Many of the sporelings soon became loosened from the slide. The length of the sporeling on the seventh day was 0.08 mm. As the oospore is about 0.072 mm in diameter, the sporeling had increased 0.008 mm in length in seven days. For a graphic representation of this increase in length see plate 52.

The effect of different pH values and temperatures on the size of sporelings as measured by width and length at the end of four weeks is summarized in table 4, from which the following is evident:

TABLE 4. *Size of sporelings in different pH values and different temperatures of water at the end of 4 weeks.*

pH	10.5°C-13°C		11°C-17°C		11°C-24°C		11°C-30°C	
	width	length	width	length	width	length	width	length
8.8	0	0	0	0	0	0	0	0
8.6	90	320	90	214	69	123.5	0	0
8.4	90	384	90	396	78	217.5	78	116
8.0-8.2	96	640	112	680	89	288	80	168
7.8	88	496	92	336	84	288	76	128
7.6	90	502	90	256	69	208	76	96
7.4	72	388	90	272	75	276	0	0
7.2	76.8	416	90	208	72	144	76	102
7.0	76	320	78	176	78	88	0	0
6.8	76	240	76	128*	78	81	0	0
6.6	76	208	76	160	0	0		

* Dead on 24th day.

1. The maximum growth is found in seawater having a temperature not higher than 17°C.

2. The largest plants as a rule are found in seawater having a pH value of 8.0-8.2, in any of the temperatures considered.

3. The growth in width of the sporeling is small in comparison with the growth in length. The greatest growth in width is found where the temperature of the water is not higher than 17°C, and where the pH value of the water is 8.0-8.2. Plate 51 shows a graphic representation of the size of the sporelings as affected by the different temperatures and the various pH values. It will be observed that in this particular set the sporelings made little or no growth where the temperature ranged from 11°-17°C and the water had a pH value of 6.8. The sets were all run in duplicate and there was a more normal growth in the corresponding set of the same pH.

Study of beaches

In the case of beaches having neither Ulva nor Fucus and only smooth rolling stones for attachment, some other factor or factors than that of the pH value of the water must be responsible since the average pH value of the water at such places was 8.07, which is well within the limits for an abundant growth of Fucus. Experiments previously conducted by the writer (Gail, 1918) threw some light on the situation. It was then believed that desiccation was the limiting factor. Considering the evidence presented in this paper, it is now considered that temperature is also an important factor.

As during the previous summer, oospores were planted on smooth flat stones which were firmly attached to the beach, a 10 per cent germination resulted on two different occasions when the temperatures did not go above 19° or 20°C. At another time scarcely a 1 per cent germination resulted, when the temperature remained at 26°C for nearly 4 hours. In one case all of the sporelings disappeared in five days. In another trial they disappeared in eight days. In the last trial, during which it was more cloudy than usual, most of the sporelings remained on the stones until the thirteenth day. The temperature in the first case went up to 24°, 24.5° and 26°C, during the first three days. When the sporelings remained on the stones eight days the temperature rose to 26°C on two different days. but only for about an hour on each occasion. In the last case the temperature did not go higher than 20°C until the last two days, when it rose to 25°C and 28°C, respectively. The temperature of 28°C was maintained for over two hours.

In order to determine whether desiccation or temperature was the limiting factor experiments were started as follows. Oospores were

planted on glass slides and placed in three different glass jars each containing two liters of seawater having a pH value of 8.2. The temperature of one jar did not go higher than 13°C. In a second jar it did not go higher than 17°C. In a third jar it did not go above 27°C. Each jar contained two slides and each slide contained over 75 oospores. A 95 per cent germination took place in those jars in which the temperature varied from 11° to 17°C. About a 3 per cent germination resulted in those jars in which the temperature went as high as 27°C, when this high temperature was maintained 2 hours or more. In these experiments the desiccation factor was eliminated and the effect of temperature was clearly demonstrated. Undoubtedly desiccation on the beach is harmful to the germination of oospores and the growth of sporelings but the high temperatures often reached may be the determining factor if they continue any considerable length of time.

Study of tidepools

A study of tidepools was also made in order to find why Fucus does not grow in them. Chambers (1912) claims that young plants of *Prionitis lyallii* are always found starting around the rim of tidepools where the CO_2 would supposedly be abundant, but never on the bottom, where both CO_2 and oxygen would in all probability be absent or much diminished. He believes the whole problem would resolve itself into a question of aeration. The writer has made numerous tests in tidepools and found that the average pH value of the seawater in them between 1:00 P. M. and 4:00 P. M. was 8.59, and that it was often 8.8. This is partly brought about by the seawater draining from the surrounding Fucus beds into the tidepools when the tide is going out. As the seawater makes its way down into the tidepools, it is continually taking up oxygen and is well aerated. The microscopic algae and *Prionitis lyallii* also make the water more alkaline in the manufacture of their carbohydrates. During the night CO_2 is liberated largely and oxygen is used in respiration. This CO_2 unites with H_2O, forming H_2CO_3, which makes the seawater in the tidepools more acid. The average pH value was 7.43 a little before sunrise. The writer has never found a good growth of Fucus in seawater having as high pH values as occur in tidepools during the day nor in seawater having as low pH values as occur in tidepools in the early morning.

The average temperature of the tidepools between 1:00 P. M. and 4:00 P. M. was 24.7°. This high temperature has already been shown to be harmful to Fucus. When oospores were planted on shells and placed in the tidepools there was less than a 5 per cent germination. Those that did germinate soon became loosened from the shells. The

only conditions of the tidepools measured by the writer that were different from those of the surrounding Fucus beds were temperature and the pH values of the seawater in the tidepools. In the light of this investigation, these two factors are believed to prevent the growth of Fucus in tidepools.

Depths at which Fucus grows below surface of water

It was shown by experiment (Gail, 1918) that the oospores, sporelings, young and mature plants of Fucus died, or that decomposition took place, when suspended in the seawater of the Sound at a depth greater than 1 meter. The average pH value of the seawater taken the following summer at the same location and at a depth of nearly 1 meter was 8.0. This is well within the limits for a good growth of Fucus. The pH value of the seawater, however, does decrease with the depth. This would also indicate a low oxygen content. At the close of the season of 1918, light was believed to be a controlling factor, and is still so regarded. This is in accord with Davis (1911) who says "The depth to which certain algae may descend depends upon the penetration of light." The writer now considers that the pH value and the low oxygen content of the seawater at the greater depths may also be important factors. Lacking facilities for measuring the pH value and the oxygen content at any great depth, no accurate measurements could be made.

Summary

1. The growth of sporelings as well as larger plants of Fucus is almost completely inhibited in seawater having a higher pH value than 8.6, and is very much inhibited in seawater having a higher pH value than 8.4.

2. Sporelings as well as larger plants of Fucus will not live in seawater having a pH value of 8.6 when the temperature is higher than 24°C.

3. Sporelings as well as larger plants of Fucus are very much inhibited in growth when the pH value of the water is below 7.2. Neither will live in seawater having a pH value of 7.0 when the temperature is above 24°C.

4. Fucus is not found on beaches, even tho there be good attachment, where there is much growth of Ulva, since Ulva causes the seawater to have too high pH values.

5. The results of the experiments on permeability of the oospores indicate that the plasma membrane is sufficiently permeable to OH and H ions in seawater having pH values above 8.4 and below 6.8 to reduce the percent of germination and to inhibit the growth of sporelings.

6. Fucus is not found on smooth gravel on beaches even where Ulva is not present, since the high temperatures and extreme desiccation decrease the germination and prevent the growth of sporelings.

7. The oospores of Fucus in seawater having a pH value of 7.0 do not germinate if the the temperature is as high as 30°C for three hours or longer. Germination is retarded at lower temperatures in seawater having pH values below 7.2.

8. Fucus is not found in tidepools because the temperature of the water is too high and because the extremes of the pH values of the water are too far apart.

9. Reduced light is a controlling factor in determining the lower
~~tors.~~
limit of Fucus. The probable low pH value and low oxygen content of the seawater at any considerable depth may also be important fac-
tors

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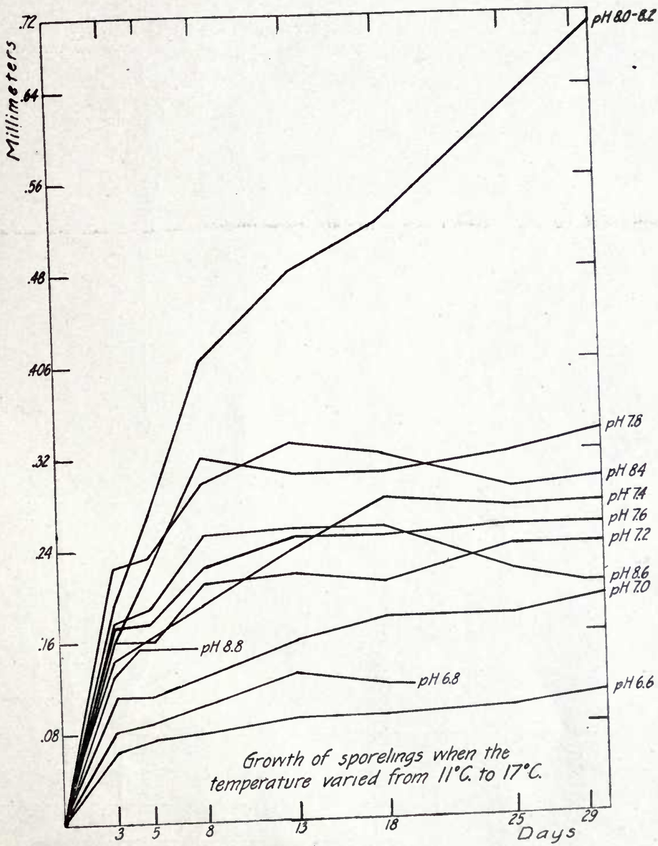

Millimeters
.72
.64
.56
.48
.406
.32
.24
.16
.08
pH 8.0-8.2
pH 7.8
pH 8.4
pH 7.4
pH 7.6
pH 7.2
pH 8.6
pH 7.0
pH 8.8
pH 6.8
pH 6.6
Growth of sporelings when the temperature varied from 11°C. to 17°C.
3
5
8
13
18
25
29
Days

PLATE 51

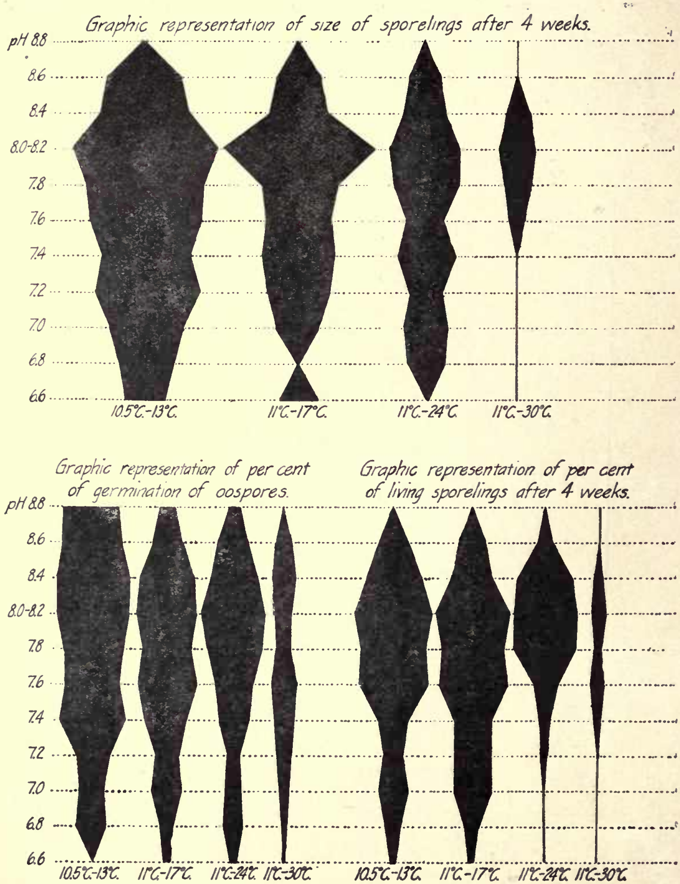
Graphic representation of size of sporelings after 4 weeks.
pH 8.8
8.6
8.4
8.0-8.2
7.8
7.6
7.4
7.2
7.0
6.8
6.6
10.5°C.-13°C.
11°C.-17°C.
11°C.-24°C.
11°C.-30°C.
Graphic representation of per cent of germination of oospores.
Graphic representation of per cent of living sporelings after 4 weeks.
pH 8.8
8.6
8.4
8.0-8.2
7.8
7.6
7.4
7.2
7.0
6.8
6.6
10.5°C.-13°C.
11°C.-17°C.
11°C.-24°C.
11°C.-30°C.
10.5°C.-13°C.
11°C.-17°C.
11°C.-24°C.
11°C.-30°C.

PLATE 52